Polychlorinated Hydrocarbon-Induced Proliferation of Maxillary and Mandibular Squamous Epithelia

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ABSTRACT

Polychlorinated hydrocarbons, including polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous environmental contaminants that bioconcentrate in the food chain. Numerous studies have demonstrated mink (*Mustela vison*) to be one of the most sensitive species to TCDD, commercial PCB mixtures, environmentally derived mixtures of PCBs and PCDDs and individual PCB congeners. In a recent study, we observed an unexpected and unique lesion, maxillary and mandibular osteoinvasive periodontal squamous cell proliferation in 12-week-old mink fed a diet containing 24 μg 3,3',4,4',5-pentachlorobiphenyl (PCB 126)/kg feed. A subsequent study in our laboratory indicated that the lesion could be induced in mink by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at a dietary concentration of 2.4 *u*g/kg feed. Numerous reports in the literature suggest that the PCB 126/TCDD-induced proliferation of maxillary and mandibular squamous epithelia that we observed in mink may be occurring in a number of different animal species, including humans, at environmentally relevant concentrations. Because of this possibility, the first objective of the present study was to determine if proliferation of maxillary and mandibular squamous epithelia could be induced in ranch mink exposed to environmentally relevant concentrations of polychlorinated hydrocarbons (PCBs, PCDDs and PCDFs) in utero, during lactation and throughout the growth period. The second objective was to determine if the lesion could be induced in a typical laboratory species, such as the mouse, which would enhance the probability of confirming the lesion's origin and examination of the mechanism of action using molecular biological techniques. Adult female mink were fed diets containing 0, 20, 40 or 60% of a Saginaw River

fish/water blend that provided 0.03, 0.83, 1.05 and 1.69 mg total PCBs/kg feed, respectively, prior to breeding through weaning of the resulting offspring. Mink kits were maintained on their respective diets for up to 27 weeks of age. At six and 27 weeks of age, six to eight kits in each treatment group were necropsied and their jaws examined for evidence of maxillary and mandibular squamous cell proliferation. Results indicated that the lesion was apparent in four of seven 27-week-old kits in the 1.05 mg total PCBs (47.6 ng TEQs/kg)/kg feed group and six of the eight kits in the 1.69 mg total PCBs (73.2 ng TEQs/kg)/kg feed group. Initial cysts in the molar region were lined with thick layers of stratified squamous epithelium and filled with floating, sloughed squamous cells. Lesions in the pre-molar, canine and incisor region of the jaw were characterized as multiple nodules of compact stratified squamous epithelium. Adult female mice were fed diets containing 5.0, 10 or 20 *u*g PCB 126/kg feed or 0.5, 1.0 or 2.0 *u*g TCDD/kg feed beginning 45 days prior to breeding and continuing through weaning of offspring. Mouse pups were maintained on their respective treatment diets for up to 98 days of age. At 21 days of age, six pups per treatment were necropsied and the mandibles and maxillae examined for evidence of squamous cell proliferation. At 28 days of age and every two weeks thereafter through 98 days of age, two pups per treatment were necropsied and the jaws examined. Histological changes were detected in the maxillae and mandibles of offspring exposed to 20 *u*g PCB 126/kg feed and in pups exposed to 0.5, 1.0 and 2.0 *u*g TCDD/kg feed. The histological changes consisted of one or more aggregates of two to five epithelial cells in the periodontal ligament of the molars. The occurrence of the aggregates appeared to be treatment-related, but there was not a clear relationship between aggregate occurrence and length of exposure. There was no evidence of

osteolysis and tissue damage in any of the examined jaws. The results of the mink trial indicated that proliferation of maxillary and mandibular squamous epithelia can be induced by consumption of diets containing environmentally relevant concentrations of PCBs, PCDDs and PCDFs. Thus, it is apparent that wild mink inhabiting areas contaminated with polychlorinated hydrocarbons are at risk of developing a lesion that could lead to eventual loss of teeth. Results of the mouse trial indicate histological changes in the maxillae and mandibles of animals exposed to PCB 126 and TCDD, but the aggregates of epithelial cells did not appear to be similar in form to the nests of squamous epithelial cells observed in the mink. Because there was no proliferation of aggregates with subsequent osteolysis and tissue damage with increasing time of exposure, the mouse is not considered to be a viable animal alternative to the mink in terms of mandibular and maxillary squamous cell proliferation.

INTRODUCTION

Polychlorinated hydrocarbons, including polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous environmental contaminants that bioconcentrate in the food chain (Tillitt *et al*., 1996) have been deemed a threat to human and wildlife health (Couture *et al*., 1990). Within this class of structurally related chemicals, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic whereas the environmentally prevalent PCB congener 3,3'4,4',5-pentachlorobiphenyl (PCB 126) is 0.1 as toxic as TCDD (Safe, 1990). These chemicals act via a common mechanism that involves binding of the toxicant to a protein located within the cytosol of the cell, the aryl hydrocarbon receptor (AhR), and translocation of the toxicant-AhR complex within the cytosol to the nucleus of the cell where it binds to specific sites on the DNA molecule. The binding of the toxicant-AhR complex with DNA alters gene expression that leads to characteristic pleiotropic responses, including cell proliferation (Whysner and Williams, 1996).

Numerous studies have demonstrated mink (*Mustela vison*) to be one of the most sensitive species to TCDD, commercial PCB mixtures, environmentally derived mixtures of PCBs and PCDDs and individual PCB congeners (Aulerich *et al*., 1971, 1973, 1985, 1986, 1987, 1988; Aulerich and Ringer, 1977; Bleavins *et al*., 1980; Hornshaw *et al*., 1983, 1986; Hochstein *et al*., 1988, 1998; Giesy *et al*., 1994; Heaton *et al*., 1995a, 1995b; Tillitt *et al*., 1996; Restum *et al*., 1988; Shipp *et al*., 1998a, 1998b; Halbrook *et al*., 1999), making it a preferred species for ecological risk assessments (Giesy *et al*., 1994). In a recent study (Render *et al*., 2000a), we observed an unexpected and unique lesion, maxillary and mandibular osteoinvasive periodontal squamous cell proliferation in 12-

week-old mink fed a diet containing 24 μg PCB 126/kg feed. Over the course of the 69-day exposure period, all 20 mink developed the lesion. Gross examination revealed mandibular and maxillary lesions consisting of swelling of the lower and upper jaws with nodular proliferation of the gingiva and loose teeth with increased gingival surface area. Radiographs indicated periodontal osteolysis of the maxilla and mandible. Histological examination documented extensive osteoinvasive periodontal squamous cell proliferation that appeared to originate from epithelial rests of Malassez adjacent to the teeth. A subsequent study (Render *et al*., 2001) in which six- and 12-week-old mink were fed 24 μg PCB 126 or 2.4 μg TCDD/kg feed, verified induction of the lesion (maxillary and mandibular osteoinvasive periodontal squamous cell proliferation) by PCB 126, demonstrated that it is also induced by TCDD, and indicated that the lesion could be detected histologically after only two weeks of treatment. Displacement of the incisor and canine teeth were observed grossly by day 28 in the six-week-old PCB- and TCDD-treated animals and by day 15 in the 12-week-old PCB- and TCDD-dosed mink. Examination of adult female mink that were fed 5.0 μg TCDD/kg feed for six months indicated no gross abnormalities of the maxilla or mandible, but histologically there was proliferation of periodontal squamous epithelial cells (Render *et al*., 2000b). Nests of squamous epithelium were present adjacent to the teeth and some had cystic centers. The proliferation resulted in focal loss of alveolar bone or osteolysis but not to the extent that was observed in six- and 12-week-old mink fed PCB 126 or TCDD. These results suggested that juvenile mink exposed to PCB 126 or TCDD are more susceptible to proliferation of periodontal squamous epithelium than adult mink.

The jaw lesion that we have observed in mink in a laboratory situation appears to have some similarities to lesions reported in human Yusho victims and lesions reported in marine mammals collected from locations having high concentrations of organochlorine compounds including PCBs. Exostosis and osteolysis of mandibular alveolar bone and loss of teeth have been reported in harbor seals (Mortenson *et al*., 1993) and gray seals from the Baltic (Bergman *et al*., 1992). A high incidence of periodontitis with necrosis and loss of teeth, believed due to exposure to environmental contaminants, including PCBs, has been reported in beluga whales from the St. Lawrence Estuary (Beland *et al*., 1993; DeGuise *et al*., 1995). Gingival hyperplasia was reported as a common clinical sign in human neonates exposed to PCBs and related compounds in Yusho, Japan in 1968 (Yamashita and Hayashi, 1985). A recent case report (Shimizu *et al*., 1992) indicated that a 24-year-old woman who had ingested rice bran oil contaminated with PCBs and related compounds at six years of age during the Yusho incident had periodontal disease because of horizontal alveolar bone resorption and a deep periodontal pocket, despite good plaque control. These reports suggest that the PCB 126/TCDD-induced proliferation of maxillary and mandibular squamous epithelia that we observed in mink may be occurring in a number of different animal species, including humans, at environmentally relevant concentrations.

The first objective of the present study was to determine if proliferation of maxillary and mandibular squamous epithelia could be induced in ranch mink exposed to environmentally relevant concentrations of polychlorinated hydrocarbons (PCBs, PCDDs and PCDFs) in utero, during lactation and throughout the growth period. The source of polychlorinated hydrocarbons was fish collected from the mouth of the Saginaw River.

The second objective was to determine if the lesion could be induced in a typical laboratory species, such as the mouse, which would enhance the probability of confirming the lesion's origin and examination of the mechanism of action using molecular biological techniques.

METHODS

Mink Trial

Approximately 700 kg of carp (*Cyprinus carpio*) were collected from the Saginaw River by electroshocking on the 17 October 2000 and 26 October 2000 with assistance from personnel from the Bay City Operations Service Center of the Michigan Department of Natural Resources. After collection, fish were transported to the Michigan State University (MSU) Experimental Fur Farm and stored in a walk-in freezer (-7° C) until further processing. Fish were then ground (1.1 cm face plate), blended into a homogeneous mixture in a paddle mixer (15 minutes) and then refrozen in plastic-lined 18.9-liter containers. "Control" fish consisted of Atlantic Ocean herring (*Clupea harengus*) purchased from Landmark Foods (Dallas, TX). Ocean herring was chosen because it, like carp, is among those species of fish that contain the enzyme thiaminase. If mink are fed raw fish containing thiaminase, thiamine is destroyed resulting in thiamine deficiency or Chastek paralysis. The problem can be corrected by cooking fish at 83°C for five minutes or by providing a thiamine supplement (National Research Council, 1982). In the present study, fish were cooked to inactivate thiaminase. The herring was ground, blended and refrozen as described above. The ground carp and herring were transported frozen to Scholten Fur Farm (Wayland, MI) for inactivation of thiaminase by heating. Just prior to heating, an equal weight of water was added to the

ground fish blend to facilitate processing. Immediately after heating at 83° C for five minutes, the fish/water mixture was poured into plastic molds and frozen in a walk-in freezer (-7° C). Once transported back to the MSU Experimental Fur Farm, the herring and carp were stored frozen until preparation of the treatment diets.

The treatment diets (Table 1) were based on the MSU Experimental Fur Farm ranch diet formulated to meet the nutritional requirements of mink (National Research Council, 1982). Each diet contained 60% of the cooked fish blend, which consisted of equal portions of ground fish and water. Thus, the diets contained the equivalent of 30% fish, which is the approximate quantity of fish consumed by mink in the wild (Heaton *et al*., 1995a). The control diet contained 60% of the ocean herring blend and the remaining three treatment diets contained a mixture of the ocean fish and Saginaw River fish blends such that the percentages of Saginaw River fish blend were 20, 40 and 60% of the diet. Analysis of the diets for total PCBs indicated concentrations of 0.03, 0.83, 1.05 and 1.69 mg total PCBs/kg feed for the control and 20, 40 and 60% Saginaw River fish blend diets, respectively. The fish blend was added to the mixer first, followed by vitamin and mineral premixes, supplemental vitamin E and biotin with a 5-minute mixing interval. Cereal was added next with another 5-minute mixing interval. The remaining ingredients were then added with a 15–minute mixing interval. Near the end of the 15-minute mixing interval, four grab samples consisting of six sub-samples per grab sample were collected. Two grab samples to be used for contaminant analysis were placed in I-Chem® jars (I-Chem, New Castle, DE) and two grab samples to be used for nutrient analysis were placed in Whirl-Pak® bags (Nasco, Fort Atkinson, WI). All diet samples were stored in an ultra-cold freezer (-78° C) until analysis. One sample of each treatment diet

was shipped on dry ice to Health Research, Inc. (Rensselaer, NY) for contaminant analysis (total PCBs and non-ortho and mono-ortho PCB, PCDD and PCDF congeners). A second sample of each treatment diet was shipped on dry ice to Litchfield Analytical Service (Litchfield, MI) for nutrient analysis. The treatment diets were packed in labeled 4-liter containers that were stored in the walk-in freezer. Twenty-four hours prior to use, containers were transferred from the walk-in freezer to a walk-in cooler (4° C) to allow the feed to thaw. One container was sufficient to feed a group of 10 mink for one day.

Forty first-year (virgin), natural dark, female mink from the MSU Experimental Fur Farm herd were randomly assigned on 11 February 2002 to the four treatment groups (10 mink/group). Untreated, natural dark, male mink were used for breeding purposes only.

Mink were housed individually in wire cages (76 cm L x 61 cm W x 46 cm H). A wooden nest box (38 cm L x 28 cm W x 27 cm H) bedded with aspen shavings and excelsior ("wood wool") was attached to the outside of each cage. Cages were suspended above ground in an open-sided shed. Food and water were available *ad libitum*. Housing of animals exceeded guidelines specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 1995).

Mink were placed on their respective treatment diets beginning 12 February 2002. Animals were observed daily to assess health status. Body weights were recorded on the first day of the trial and on 28 February 2002 just prior to breeding.

The females were mated to untreated males between 1 March and 18 March 2002. Each female was given an opportunity to breed every fourth day until a successful mating was obtained. All matings were verified by the presence of "normal" appearing, motile

spermatozoa in vaginal aspirations collected immediately after copulation. Mated females were given an opportunity for additional matings (with different males) the day following the initial mating and on the eighth and ninth days after the first successful mating (a common commercial mink breeding practice).

The whelping period began on 26 April 2002 and ended on 11 May 2002. Nest boxes were checked on a daily basis for the presence of mink kits. Live and stillborn young were counted at birth and their gender determined. Body weights of the adult females were recorded at the time their litters were weighed.

Six kits from each treatment group were euthanized with CO_2 and necropsied at approximately six weeks of age on 11 June 2002. Surviving adults were euthanized and necropsied after weaning of kits from 17 June to 8 July 2002 (approximately 135 days on trial). The brain, liver, kidneys, spleen, heart, adrenal glands and thyroid gland (kits only) were removed and weighed. Samples of the liver from kits were either placed in I-Chem® jars and frozen on dry ice for subsequent contaminant analysis or in cryogenic vials (Corning CoStar Corporation, Cambridge, MA) and frozen in liquid nitrogen for subsequent determination of microsomal enzyme activities. The heads of the six-week-old kits were placed in a 10% formalin-saline solution (10% formalin in 0.9% sodium chloride) for subsequent histological examination of the mandibles and maxillae. Skulls were decalcified in 5% nitric acid, rinsed, trimmed, processed using a routine histotechnologic method and embedded in paraffin. Tissues were sectioned at six microns and stained with hematoxylin and eosin. The lesion was graded as mild, moderate or severe based on the number and size of foci of squamous cell proliferation in the maxilla and mandible.

Eight kits from each treatment group were maintained on their respective treatment diets. On 5 November 2002, these kits (approximately 27 weeks old) were necropsied with tissues being handled as described above.

Mouse Trial

Treatment diets contained 5.0, 10 or 20 μg 3,3',4,4',5-pentachlorobiphenyl (PCB 126)/kg feed or 0.5, 1.0 or 2.0 μg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg feed. PCB 126 (AccuStandard, Inc., New Haven, CT; 100% pure) was purchased in a 5 mg quantity that was subsequently dissolved in hexane to provide a stock solution of 1.67 mg/ml. Appropriate quantities of the PCB 126 stock solution were diluted in corn oil (100 ml final volume) and then added to 1 kg of ground Harlan Teklad 22/5 Rodent Diet (Madison, WI) to prepare the dietary premixes. The premixes were then combined with the appropriate quantities of ground rodent diet to give the desired PCB 126 dietary concentrations and thoroughly mixed for 10 minutes. TCDD (AccuStandard, Inc., New Haven, CT; 99.1% pure) was purchased as a solution (50 *u*g/ml toluene). Appropriate quantities of the TCDD solution were diluted in corn oil (100 ml final volume) and added to ground rodent chow to prepare dietary premixes as described above. Preparation of the final diets was as described for the PCB 126 diets. Control feed was prepared as described above with the exception that only corn oil was added to the rodent chow.

Thirty-two non-pregnant, proven breeder CD-1 mice (Harlan, Indianapolis, IN) were delivered to MSU's University Laboratory Animal Resources (ULAR) Clinical Center animal containment facility on 7 October 2001. Animals were housed individually in disposable mouse boxes and provided feed (Harlan Teklad 22/5 Rodent Diet) and water *ad libitum*. Temperature and humidity were maintained at 22° C and 45%, respectively.

The photoperiod was 12 hours light and 12 hours dark. On 25 October 2001, mice were randomly assigned to one of the seven treatment groups (eight mice in the control group and four mice in each of the PCB 126 and TCCD groups) and weighed. The ground feed containing PCB 126 or TCDD was provided in porcelain cups with stainless steel followers. Animals were checked daily and weighed weekly. On 2 December 2001, 32 male CD-1 mice (Harlan) were delivered to the ULAR Clinical Center animal containment facility. These animals were housed individually and provided untreated feed and water *ad libitum*. After one week of acclimation, a male was introduced into each female's cage until the female was judged to be pregnant by casual observation. At parturition, the mouse pups were counted and their sex determined. When pups were 21 days of age, they were weaned from the dam and housed with littermates of the same sex. At 21 days of age, six pups (three males and three females) from each of the seven treatment groups were euthanized with sodium pentobarbital (86 mg/kg body weight; Abbott Laboratories, North Chicago, IL) and weighed. The head was removed, placed in a Whirl-Pak® bag (Nasco, Fort Atkinson, WI) on ice and immediately delivered to MSU's Animal Health Diagnostic Lab (AHDL) for further processing, as described for the mink. Beginning at 28 days of age and every two weeks thereafter through 98 days of age, two pups (one male and one female) from each treatment were euthanized, weighed and head collected and processed as described above.

Statistical analyses were performed using SAS® software (SAS; Statistical Analysis Systems, Release 8.0, Cary, NC). Statements of significance are based on $p < 0.05$.

RESULTS

Mink Trial

Concentrations of the individual non-ortho and mono-ortho PCB congeners and PCDD and PCDF congeners in the diets and the toxic equivalents (TEQs) contributed by them are presented in Table 2. The concentrations of TEQs ranged from 2.3 ng/kg in the control diet to 73.2 ng/kg in the diet containing 1.69 mg total PCBs/kg feed. The percent contribution by non-ortho PCBs, mono-ortho PCBs, PCDDs and PCDFs to the total TEQ concentration in the treatment diets averaged 25.4%, 18.3%, 32.3% and 23.9%, respectively. Almost 50% of the TEQs were contributed equally by 3,3'4,4'5-pentachlorobiphenyl (PCB 126) and 2,3,4,7,8-pentachlorodibenzofuran.

Consumption of diets containing PCBs, PCDDs and PCDFs derived from Saginaw River fish had no effect on breeding success (number of females bred/total number of females) or whelping success (number of females whelping/number of females bred) of female mink (Table 3). Gestation length was not significantly affected by exposure to PCBs, PCDDs and PCDFs (Table 4).

The average litter size was similar across all four treatment groups (5.7, 5.8, 5.8 and 6.3 kits for females in the control and 0.83, 1.05 and 1.69 mg total PCBs/kg feed groups, respectively). There were no significant differences in kit survivability from birth to six weeks of age (Table 5).

Body weights of adult females were not significantly affected by consumption of PCBs, PCDDs and PCDFs derived from Saginaw River fish during the course of the trial (Table 6). Similarly, body weights of male and female kits in the four treatment groups

did not differ significantly from one another from birth through 27 weeks of age (Tables 7 and 8).

The absolute and relative (expressed as a percentage of body weight) brain, liver, kidney, heart and adrenal gland weights of the adult females were not significantly different between treatment groups at necropsy (Tables 9 and 10). Absolute and relative spleen weights of females fed the diet containing 0.83 mg total PCBs/kg feed diet were significantly greater compared to the control group. In kits necropsied at six weeks of age, there were no significant differences in absolute and relative organ weights (Tables 11 and 12). Male and female relative adrenal gland weights are presented separately because there was a significant treatment by sex interaction, but there were no differences between treatments within sex (Table 12). Relative and absolute spleen weights of 27-week-old kits in the 1.69 mg total PCBS/kg feed group were significantly greater compared to controls (Tables 13 and 14).

Histological evidence of mandibular and/or maxillary squamous cell proliferation was apparent in four of seven 27-week-old kits in the 1.05 mg total PCBs (47.6 ng TEQs/kg)/kg feed group and six of the eight kits in the 1.69 mg total PCBs (73.2 ng TEQs/kg)/kg feed group (Table 15**)**. The lesions in the 1.05 mg total PCBs/kg feed group were considered mild in all four kits. These animals had one or two cystic lesions that tended to be located in only one quadrant of the jaw, with the exception of 1400-F2-28 that had a focal cyst each in the left and right halves of the maxilla. The cysts were consistently located in the molar region of the jaws in all four animals. The lesions in the 1.69 mg total PCBs/kg feed group were rated mild to moderate. Mink in this treatment group had up to 10 foci dispersed throughout two to four quadrants of the jaw in the

incisor, canine, pre-molar and molar regions. Initial cysts in the molar region were lined with thick layers of stratified squamous epithelium and filled with floating, sloughed squamous cells. Lesions in the pre-molar, canine and incisor region of the jaw were characterized as multiple nodules of compact stratified squamous epithelium.

Mouse Trial

All but two females (one in the 20 *u*g PCB 126/kg feed group and one in the 1.0 *u*g TCDD/kg feed group) had litters (Table 16). The average litter size across all treatments was similar, ranging from 11.3 pups in the 1.0 *u*g TCDD/kg feed group to 14.8 pups in the 5 *u*g PCB 126/kg feed group. The control group had an average litter size of 12.1 pups. Survivability of pups from birth to weaning at 21 days of age was not significantly affected by exposure to PCB 126 or TCDD, although survivability in the 2.0 μg TCDD kg/feed group was 52% lower than survivability in the control group (42% versus 94%, respectively (Table 16).

Examination of mouse jaws indicated that there was no evidence of histopathological changes in the maxillae and mandibles of the adult female mice that were evaluated. The duration of exposure of the dams to PCB 126 or TCDD averaged 102 days (45 days prior to breeding through the 21-day lactation period). Histological changes were detected in the maxillae and mandibles of the resulting offspring exposed to PCB 126 or TCDD from conception until necropsy at 21 days through 98 days of age (Table 17). The histological changes consisted of one or more aggregates of two to five epithelial cells in the periodontal ligament of the molars. The occurrence of the aggregates appeared to be treatment-related because they were not detected in the control animals that were examined. Four of the five animals exposed to 20 *u*g PCB 126/kg feed expressed foci of

epithelial cell aggregates. These animals ranged in age from 21 to 84 days. A 42-day-old animal in this group had no detectable aggregates. The occurrence of aggregates was also determined in mice representing all three TCDD treatment groups. In general, the number of foci per animal increased as the dietary TCDD concentration increased. There was not a clear relationship between aggregate occurrence and length of exposure in that the histological change was detected in 28-day-old animals in the 1.0 and 2.0 *u*g TCDD/kg feed groups, but not in 84-day-old animals on those two diets. There was no evidence of osteolysis and tissue damage in any of the examined jaws.

DISCUSSION

Mink Trial

Fish collected from the Saginaw River contained a variety of PCB, PCDD and PCDF congeners, as indicated by their presence in the treatment diets (Table 2). Complex mixtures of environmental contaminants such as this can make toxicity assessment difficult because of the number of different components and the potential synergistic and antagonist relationship between the components. However, several of the planar PCB, PCDD and PCDF congeners cause a set of toxic responses very similar to those caused by TCDD, the most toxic of these chemicals (Safe, 1990). The toxic responses result from the initial binding of the TCDD-like congeners to the AhR, translocation of the AhR-ligand complex into the cell nucleus and activation of specific genes. Enhanced expression of these genes leads to the pleiotropic responses associated with exposure to TCDD (Safe, 1990). Because the TCDD-like PCB, PCDD and PCDF congeners act by a common mechanism (binding to the AhR), the toxicity of complex mixtures containing these congeners can be assessed by using the toxic equivalency

factor (TEF) approach. In using this approach, the potency of the individual congener is expressed relative to the potency of TCDD based on the dose required to produce a specific response. The toxicity of a complex mixture of congeners acting by the same mechanism can be assessed by determining the product of the concentration of each congener and its TEF value to give the concentration of the TCDD toxic equivalents or TEQs contributed by each congener. The TEQs contributed by each congener can be summed to give the total concentration of TEQs present in the complex mixture. Determination of the concentration of TEQs present in, for example, a food source provides a more accurate assessment of the quantity of toxic material ingested rather than considering only total PCB concentration.

The concentrations of total PCBs in the different treatment diets (Table 2) were comparable to dietary PCB concentrations in a study of similar design conducted by Heaton and associates (1995a). In that study, mink diets contained 0, 10, 20 and 40% carp collected from Saginaw Bay with total PCB concentrations of 0.015, 0.72, 1.53 and 2.56 mg/kg feed, respectively. In the present study, diets containing 0, 20, 40 and 60% of the Saginaw River fish/water blend, which corresponds to 0, 10, 20 and 30% Saginaw River fish, had total PCB concentrations of 0.03, 0.83, 1.05 and 1.69 mg/kg feed, respectively. Total TEQ concentrations in the present study (2.3, 27.6, 47.6 and 73.2 ng TEQs/kg in the control and 0.83, 1.05 and 1.69 mg total PCBs/kg feed diets, respectively) were comparable to dietary TEQ concentrations in the Heaton *et al*. (1995a) study as reported by Tillett *et al*. (1996) using TEF values presented in Van den Berg *et al*. (1998) (1.0, 22.2, 43.0 and 85.0 ng/kg in the control and 0.72, 1.53 and 2.56 mg total PCBs/kg feed diets, respectively). The percentages of TEQs contributed by non-ortho PCBs,

mono-ortho PCBs, PCDDs and PCDFs were somewhat different between the two studies. In the present study, PCDDs and PCDFs both contributed 27.6% of the TEQs while non-ortho and mono-ortho PCB congeners contributed 26.1 and 18.6%, respectively. In the Heaton *et al*. (1995a)/Tillett *et al*. (1996) study, using TEF values presented in Van den Berg *et al*. (1998), the majority of TEQs were contributed by non-ortho PCBs (45.9%) and mono-ortho PCBs (27.5%), while PCDDs and PCDFs contributed 15.8 and 10.6%, respectively. The predominant congeners in the present study were PCB 126 and 2,3,4,7,8-pentachlorodibenzofuran based on TEQ contribution (25.1 and 20.6%, respectively) compared to PCB 126 (48.2%) in the Heaton *et al*. (1995a)/Tillett *et al*. (1996) study, using TEFs presented in Van den Berg *et al*. (1998).

Consumption of diets containing PCBs derived from fish collected from the Saginaw River did not have an adverse effect on adult mink reproduction based on breeding success, whelping success and gestation length (Tables 3 and 4). Likewise, kit survivability from birth through six weeks of age was not different between the four treatment groups (Table 5).

Hornshaw *et al*. (1983) fed female mink a diet containing 1.5 mg total PCBs/kg feed derived from Saginaw Bay fish for seven months prior to breeding. These mink failed to whelp any kits. Restum *et al*. (1998) reported that female mink fed 1.0 mg total PCBs/kg feed had similar reproductive success as the control mink, but kit survivability at birth and at three and six weeks of age was significantly lower compared to controls. In a study by Halbrook *et al*. (1999), mink were fed diets containing up to 1.86 mg total PCBs/kg feed derived from fish collected from Poplar Creek (located on the Oak Ridge Reservation, Oak Ridge, TN) from three months prior to breeding through weaning. The

females fed the diet containing the highest concentration of total PCBs had the smallest average litter size (4.3 kits versus 6.5 kits for the controls; not significant) but kit survivability in this group through six weeks of age was the highest (91% compared to 51% for the controls; not significant). Heaton *et al*. (1995a) reported no differences in breeding and whelping success in mink fed diets containing from 0.72 to 2.56 mg total PCBs/kg feed derived from Saginaw Bay fish. However, the number of live kits whelped by females fed 2.56 mg total PCBs/kg feed (85.0 ng TEQs kg; Tillett *et al*., 1996 using TEF values presented in Van den Berg *et al*., 1998) was significantly lower compared to the control group and groups fed 0.72 (22.2 ng TEQs/kg; Tillett *et al*., 1996; Van den Berg *et al*., 1998) and 1.53 (43.0 ng TEQs/kg; Tillett *et al*., 1996; Van den Berg *et al*., 1998) mg total PCBs/kg feed (0.7, 5.0, 3.8 and 4.8 kits, respectively). Additionally, all of the kits whelped by females fed 2.56 mg total PCBs/kg feed were either stillborn or died within 24 hours. Percent kit survival to six weeks of age was 85% for the controls and 28% and 12% for kits in the 0.72 and 1.53 µg total PCBs/g feed groups, respectively.

The lack of an effect on kit survivability in the present study at dietary total PCB and TEQ concentrations that are two to three times greater than total PCB and TEQ concentrations associated with reduced survivability in the Heaton *et al*. (1995a)/Tillett *et al*. (1996) study is difficult to explain. A possible explanation could be the moderate differences in congener profile of the diets. In the present study, the diets contained relatively more TEQs contributed by PCDDs and PCDFs compared to the diets used in Heaton *et al*. (1995a)/Tillett *et al*. (1996) study. While the TEF approach assumes additivity of constituent congeners, it is possible that there are synergistic and antagonistic relationships that are not accounted for in the TEF approach, thus explaining

the apparent differences in toxicity of feed containing environmentally-derived polychlorinated compounds from the same general source (Saginaw Bay and Saginaw River fish).

Body weights of adult females and kits were not affected by consumption of feed containing PCBs, PCDDs and PCDFs derived from Saginaw River fish (Tables 6-8). In other studies conducted in a similar manner, adult body weights tended to be unaffected while kit body weights tended to decrease with increasing contaminant concentrations. Heaton *et al*. (1995a) reported that despite a dose-related PCB-induced decrease in feed consumption, body weights of adult females were not affected. Similarly, adult mink body weights were not affected in the fish-feeding study conducted by Halbrook *et al*. (1999). Both Heaton *et al*. (1995a) and Restum *et al*. (1998) reported decreased body weights of kits exposed to as little as 0.72 (22.2 ng TEQs/kg; Tillitt *et al*. 1996; Van den Berg *et al*., 1998) and 0.25mg total PCBs/kg feed derived from Saginaw Bay fish, respectively, at three and six weeks of age. Birth weights of kits were significantly decreased in the 1.53 (43.0 ng TEQs/kg, Tillett *et al*. 1996; Van den Berg *et al*., 1998) mg total PCBs/kg feed group in the Heaton *et al*. (1995a) study and in the 0.50 mg total PCBs/kg feed group in the Restum *et al*. (1998) study. Halbrook *et al*. (1999) reported that body weights of six-week-old male kits exposed to 1.86 µg total PCBs/g feed were significantly less compared to controls.

The only changes in adult organ weights was an increase in absolute and relative spleen weights of adult females fed 0.83 mg (27.6 ng TEQs/kg) total PCBs/kg feed (Tables 9 and 10). Heaton *et al*. (1995a) reported a general dose-dependant increase in relative organ weights (expressed as a percent of brain weight) with liver and spleen

weights increased at all PCB concentrations (0.72 to 2.56 mg total PCBs/kg feed), adrenal gland weights increased at 1.53 and 2.56 mg total PCBs/kg feed and kidney weights increased at 2.56 mg total PCBs/kg feed. Restum *et al*. (1998) reported increased absolute and relative (percent of brain weight) liver weights of male mink fed 1 mg total PCBs/kg feed for 18 months and an increase in absolute liver weights of males fed 0.5 mg total PCBs/kg feed over the same time period. Restum *et al*. (1998) also reported that absolute and relative spleen weights of females fed 1.0 mg total PCBs/kg feed were increased compared to controls after 18 months of exposure.

Absolute and relative organ weights of six-week-old kits were not affected by consumption of PCBs/PCDDs/PCDFs derived from Saginaw River fish (Tables 11 and 12). Absolute and relative spleen weights of 27-week-old kits exposed to 1.69 mg (73.2 ng TEQs/kg) total PCBs/kg feed were significantly greater compared to controls in the present study (Tables 13 and 14). Heaton *et al*. (1995a) reported a general decrease in six-week-old kit relative organ weights at doses of 0.72 and 1.53 mg total PCBs/kg feed. Restum *et al*. (1998) reported that mink kits exposed to PCBs for 60 weeks had increased absolute (0.25 and 1.0 mg total PCBs/kg feed) and relative (0.25, 0.50 and 1.0 mg total PCBs/kg feed) spleen weights while absolute brain (1.0 mg total PCBs/kg feed), kidney (0.50 and 1.0 mg total PCBs/kg feed) and heart (1.0 mg total PCBs/kg feed) weights were decreased.

Previous studies from our laboratory have shown that feeding PCB 126 and TCDD induces periodontal squamous proliferation in the jaws of mink. In the initial study (Render *et al*., 2000a), 12-week-old mink were fed a diet containing 24 µg PCB 126/kg feed. After 31 days on trial, one of the animals had swelling of the upper and lower jaws

with nodular proliferations of the mandibular and maxillary gingival and loose teeth. The cleaned skull of this animal had marked porosity of mandibular and maxillary bone. Histological examination of tissues from other exposed animals showed that the mucosal epithelium was thickened and mucosal epithelium adjacent to the teeth extended into the underlying bone as thin cords. The principal lesion was the presence of nests and cords of squamous epithelial cells within the periodontal ligament of multiple teeth. The nest and cords of epithelial cells extended into the adjacent alveolar bone, which was markedly irregular because of osteolysis. Large gaps occurred within the bone that corresponded with the loss of bone observed grossly. The nests of squamous epithelial cells were variable in size and some had cystic centers filled with exfoliated squamous cells. Over the subsequent 38 days of exposure, the remaining 19 mink being fed PCB 126 were affected in a similar manner.

In a subsequent study (Render *et al*., 2001), six- and 12-week-old mink were fed 2.4 µg TCDD/kg feed. Many of these animals had loose and displaced incisor teeth by day 15 (12-week-old animals) and day 28 (six-week-old animals) of exposure. Canine teeth were grossly more prominent. Radiographs showed mandibular and maxillary osteolysis of the lamina dura in exposed mink. Histologically, there was a loss of alveolar bone and solid cystic nests and cords of infiltrative epithelium in the periodontal ligament. The centers of the cysts contained exfoliated squamous cells and keratin.

Unpublished results from our laboratory indicated that 15 of 18 seven-month-old mink exposed to 0.24 µg PCB 126/kg feed in utero, during lactation and throughout the growth period had histological evidence of this lesion. While none of the mink kits had gross abnormalities of the maxilla and mandible, histologically there was proliferation of

periodontal squamous epithelial cells. Nests of squamous epithelium were present adjacent to the teeth and some had cystic centers. The proliferation resulted in focal loss of alveolar bone.

The lowest concentration of PCB 126 that resulted in the lesion in the above studies (0.24 µg PCB 126/kg feed) is approximately twice the concentration of PCB 126 in the present dietary treatments (0.11 and 0.19 µg PCB 126/kg feed in the 1.05 and 1.69 mg total PCB 126/kg feed diets; Table 2) that resulted in the lesion. If expressed on a TEQ basis, 0.24 µg PCB 126/kg feed is equivalent to 24 ng TEQs/kg feed using a TEF value of 0.1 (Van den Berg *et al*., 1998). In the present study, the diet containing 28 ng TEQs/kg (0.83 mg PCB 126/kg feed) did not induce the lesion in mink that were exposed in a manner similar to the mink in the unpublished study, but the diets containing 48 and 73 ng TEQs/kg (1.05 and 1.69 mg total PCBs/kg feed) caused squamous cell proliferation (Table 15). In the present study, the proliferation was not to the extent that it resulted in focal loss of alveolar bone or osteolysis as was reported in the previous studies (Render *et al*., 2000a, 2001). Nevertheless, the results suggest that mink exposed to environmentally relevant concentrations of PCBs/PCDDs/PCDFs are at risk in terms of maxillary and mandibular squamous cell proliferation. It is not know, at this point, if the lesion would increase in severity if the period of exposure was longer.

Mouse Trial

Consumption of diets containing up to 20 *u*g PCB 126/kg feed and 2.0 *u*g TCDD/kg feed for 45 days prior to breeding through the 21-day gestation period had no significant effect of the reproductive performance of CD-1 mice (Table 16). Courtney (1976) reported that pregnant CD-1 mice administered 10 daily doses of 25 *u*g TCDD/kg body

weight on days 7 to 16 of gestation had no change in reproductive performance compared to controls. In the present study, assuming an average body weight of 35 g and average daily feed consumption of 5 g, female mice consumed no more than 0.3 *u*g TCDD/kg body weight/day. Thus, the lack of an effect of PCB 126 and TCDD on reproduction was not unexpected. While survivability of pups through 21 days of age was not significantly affected by exposure to PCB 126 and TCDD, survivability in the 2.0 *u*g TCDD/kg feed group was considerably lower compared to the control group (42% versus 94%; Table 16). The low survivability rate can be attributed to a single female losing 11 of 15 pups four days after parturition.

Examination of mouse jaws indicated the presence of epithelial cell aggregates confined to the periodontal ligament of the molars in mice exposed to 20 *u*g PCB 126/kg feed and 0.5, 1.0 and 2.0 *u*g TCDD/kg feed from conception through 21 to 84 days of age (Table 17). Aggregates were not detected in the dams. It is possible that the squamous cell aggregates occurring in the periodontal ligament of the mouse are activated epithelial rests of Malassez (ERM). According to Spouge (1980), the original description of epithelial rests was as small circular aggregates of cells within the periodontal ligament. While the rests persist throughout life, they decrease in density with age. In a study by Wesselink and Beersten (1993), it was shown that the ERM were three to four times more frequent in the periodontal ligament along the mesial aspect of the mesial root of the mouse lower front molar than in other aspects, which is where many of the aggregates were noted in the present study. Although the precise functions of the EMR are still obscure, it is generally accepted that they are capable of reactive proliferation in response to certain environmental changes such as inflammation (Thesleff, 1987; Yamasaki and

Pinero, 1989). Thesleff (1987) showed that ERM intensively incorporated epidermal growth factor (EGF) and speculated that activation of ERM involves an increase of EGF in their environment. Abbott and Birnbaum (1998) reported that studies in mice of the mechanism through which TCDD produces cleft palate and hydronephrosis revealed correlations between effects on epithelial cell proliferation and changes in the expression of the EGF receptor, EGF and transforming factor-α. While the relationship between TCDD and the various growth factors is complicated, it is possible that in the present study in utero and lactational exposure of mice to TCDD and PCB 126 modulated the EGF receptor pathway such that activation of ERM occurred.

Although there are histological changes involving oral epithelial cells in both the mouse and the mink, the changes do not appear to be comparable in form nor in magnitude. In the mink, an island of squamous cells forms in the gingiva that quickly proliferates, leading to formation of large cysts in all quadrants of the jaw. The cells form cords that infiltrate the alveolar bone, causing osteolysis and tissue destruction, which results in tooth displacement and eventual loss. In the mouse, the epithelial cell aggregates are confined to the periodontal ligament and cause no tissue destruction, even after exposure through 84 days of age. In our initial report of mandibular and maxillary squamous cell proliferation in mink, it was suggested that the proliferating cells originated from the ERM (Render *et al*., 2000a). Support for this hypothesis came from the fact that the ERM in rats given N-methylnitrosourea were stimulated to proliferate into odontogenic neoplasms (Hamamoto *et al*., 1998) and that cells of the ERM produce a bone-resorbing factor (Birek *et al*., 1983), thus explaining the marked osteolysis. However, results of unpublished work from our laboratory and this study suggest that the

origin of the lesion observed in mink is somewhere other than the ERM, based on location of the cell nests. Thus, it is apparent that the mouse is not an acceptable animal alternative to study the mandibular and maxillary squamous cell proliferation that is so prominent in the mink.

CONCLUSION

Results of this study indicated that exposure of mink kits in utero and throughout the growth period to PCBs, PCDDs and PCDFs derived from Saginaw River fish at dietary concentrations of 1.05 mg total PCBs/kg feed (47.6 ng TEQs/kg feed) and above resulted in induction of mandibular and maxillary squamous cell proliferation. The 1.05 mg total PCBs/kg feed diet contained 40% of a fish/water bled, which was equivalent to 20% fish. Thus, it is apparent that wild mink inhabiting areas contaminated with polychlorinated hydrocarbons are at risk of developing a lesion that could lead to eventual loss of teeth resulting in death by starvation. In mice exposed to 20 *u*g PCB 126/kg feed or 0.5 *u*g TCDD/kg body weight and higher from conception through 21 to 84 days of age, small epithelial cell aggregates were detected in the periodontal ligament. Because the aggregates did not appear to be similar in form to the nests of squamous epithelial cells observed in the mink and because there was no proliferation of aggregates with subsequent osteolysis and tissue damage with increasing time of exposure, the mouse is not considered to be a viable animal alternative to the mink in terms of mandibular and maxillary squamous cell proliferation.

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Table 1. Composition and Nutrient Analysis of Experimental Diets				
Composition (%)	**Dietary Treatment (mg total PCBs/kg feed)**			
	Control	**0.83**	**1.05**	**1.69**
Saginaw River Fish	0	20	40	60
Ocean Herring [a]	60	40	20	0
Commercial Mink Cereal [b]	21.5	21.5	21.5	21.5
Duck Offal [c]	5.0	5.0	5.0	5.0
Spray-dried Chicken Liver [d]	10.0	10.0	10.0	10.0
Spray-dried Eggs [d]	2.5	2.5	2.5	2.5
Trace Mineral Premix [e]	0.5	0.5	0.5	0.5
Vitamin Premix [f]	0.5	0.5	0.5	0.5
Vitamin E [g] (IU/kg feed)	700	700	700	700
Larvadex [h] (ml/kg feed)	0.15	0.15	0.15	0.15
d-Biotin[i] (mg/kg eggs)	2.4	2.4	2.4	2.4
Nutrient Analysis (%) [j]				
Moisture	51.41	53.15	53.89	52.73
Protein	21.49	20.91	18.07	19.57
Fat	11.83	10.92	10.70	10.75
Ash	4.31	4.47	4.52	4.14
Crude Fiber	1.21	1.15	1.01	1.15
Total Digestible Nutrients	47.10	44.70	44.17	45.38

[a] Landmark Foods, Dallas, TX

[b] XK-40 Mink food, XK Mink Foods, Inc., Plymouth, WI

[c] United Feeds, Inc., Plymouth, WI

[d] Van Elderen, Inc., Martin, MI

[e] Calcium, 13.40%; copper, 2000 ppm; iodine, 30 ppm; iron, 2.00%; manganese, 2000 ppm; selenium, 60 ppm; zinc, 2.00%; Michigan State University Feed Mill, East Lansing, MI

[f] Vitamin A, 916,652 IU/kg; vitamin D_3, 91,674 IU/kg; vitamin E, 11,000 IU/kg; vitamin K activity, 2,200mg/kg; menadione, 733 mg/kg; vitamin B_{12}, 5.5 mg/kg; riboflavin, 733 mg/kg; d-panothenic acid, 2,935 mg/kg; niacin, 4,400 mg/kg; thiamine, 183 mg/kg; pyridoxine, 33 mg/kg; Michigan State University Feed Mill, East Lansing, MI

[g] α-Tocopherol, 500,000 IU/kg; Michigan State University Feed Mill, East Lansing, MI

[h] Novartis Animal Health, Greensboro, NC

[i] Archer Daniel Midland, Des Moines, IA

[j] Litchfield Analytical Services, Litchfield, MI

Table 2. Dietary Concentrations (ng/kg) of PCDDs, PCDFs, PCBs and TEQs[a].

		Dietary Treatment (mg total PCBs/kg feed)							
		Control		0.83		1.05		1.69	
Compound	**TEF[a]**	**[ ]**	**TEQs**	**[ ]**	**TEQs**	**[ ]**	**TEQs**	**[ ]**	**TEQs**
Non-Ortho PCBs									
#81	0.00010	nd	nd	nd	nd	34	0.0	60	0.0
#77	0.00010	33	0.0	395	0.0	556	0.1	816	0.1
#126	0.10000	nd	nd	72	7.2	112	11.2	188	18.8
#169	0.01000	nd	nd	nd	nd	12	0.1	21	0.2
Total Non-ortho PCBs		33	0.0	nd	7.2	714	11.4	1085	19.1
% total TEQs			0.0%		26.1%		24.0%		26.1%
Mono-Ortho PCBs									
#123	0.00010	63	0.0	577	0.1	469	0.0	1605	0.2
#118	0.00010	2408	0.2	25547	2.6	39182	3.9	67800	6.8
#114	0.00050	36	0.0	576	0.3	989	0.5	1660	0.8
#105	0.00010	901	0.1	10131	1.0	18310	1.8	27290	2.7
#167	0.00001	118	0.0	1131	0.0	1594	0.0	2730	0.0
#156	0.00050	211	0.1	2042	1.0	3451	1.7	4920	2.5
#157	0.00050	79	0.0	414	0.2	812	0.4	1050	0.5
#189	0.00010	18	0.0	234	0.0	420	0.0	590	0.1
Total Mono-ortho PCBs		3834	0.4	40652	5.2	65227	8.3	107645	13.6
% total TEQs			17.4%		18.8%		17.5%		18.6%
PCDDs									
2,3,7,8	1.00000	1.8	1.8	10	10	7.9	7.9	9.3	9.3
1,2,3,7,8	1.00000	nd	nd	0.9	0.9	4.1	4.1	7.5	7.5
1,2,3,4,7,8	0.10000	nd	nd	nd	nd	15	1.5	23	2.3
1,2,3,6,7,8	0.10000	nd	nd	nd	nd	4.4	0.4	7.4	0.7
1,2,3,7,8,9	0.10000	nd	nd	nd	nd	1.5	0.2	2.7	0.3
1,2,3,4,6,7,8	0.01000	nd	nd	1.4	0.0	5.6	0.1	7.1	0.1
OCDD	0.00010	nd	nd	3.5	0.0	15	0.0	21	0.0
Total PCDDs		1.8	1.8	15.8	10.9	54	14.2	78	20.2
# total TEQs			78.3%		39.5%		29.9%		27.6%
PCDFs									
2,3,7,8	0.10000	1.2	0.1	7.3	0.7	8.8	0.9	21	2.1
1,2,3,7,8	0.05000	0.8	0.0	4.5	0.2	10	0.5	13	0.7
2,3,4,7,8	0.50000	nd	nd	6.1	3.1	23	11.5	32	16.0
1,2,3,4,7,8	0.10000	nd	nd	1.8	0.2	2.7	0.3	5.4	0.5
1,2,3,6,7,8	0.10000	nd	nd	0.9	0.1	2.6	0.3	5.2	0.5
1,2,3,7,8,9	0.10000	nd	nd	nd	nd	nd	nd	2.1	0.2
2,3,4,6,7,8	0.10000	nd	nd	nd	nd	1.1	0.6	2.3	0.2
1,2,3,4,6,7,8	0.01000	nd	nd	1.3	0.0	3	0.1	4.2	0.0
1,2,3,4,7,8,9	0.01000	nd	nd	nd	nd	nd	nd	1.2	0.0
OCDF	0.00010	nd	nd	0.5	0.0	2.1	0.0	4.4	0.0
Total PCDFs		2	0.1	22.4	4.3	51.2	13.6	90.8	20.2
% total TEQs			4.3%		15.6%		28.6%		27.6%
Grand Total TEQs (ng/kg)			2.3		27.6		47.6		73.2

[a] nd refers to not detected

[b] Toxic equivalency factors (TEFs) are based on Van den Berg *et al*., 1998.

Table 3. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on the Number of Adult Female Mink Bred and the Number of Adult Female Mink that Whelped.

Dietary Treatment (mg total PCBs/kg feed)	Number of Females Bred / Total Number of Females	Number of Females Whelping / Number of Females Bred
Control	10/10	9/10
0.83	10/10	8/10
1.05	10/10	10/10
1.69	10/10	9/10

Table 4. The Effects of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on the Gestation Length (days) of Adult Female Mink [a].	
Dietary Treatment (mg total PCBs/kg feed)	Gestation Length
Control	48.0 ± 1.6
0.83	49.1 ± 1.7
1.05	51.5 ± 1.5
1.69	48.8 ± 1.6
[a]Data are presented as the mean ± standard error of the mean.	

Table 5. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Mink Kit Survivability (%) From Birth to Six Weeks of Age[a]	
Dietary Treatment (mg total PCBs/kg feed)	Survivability
Control	88.9 (72.1 – 98.5)
0.83	95.0 (80.6 – 100.0)
1.05	88.5 (72.5 – 98.1)
1.69	91.9 (76.4 – 99.5)
[a]Data are presented as the mean (95% confidence intervals).	

Table 6. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Body Weights (g) of Adult Female Mink[a]

Dietary Treatment (mg total PCBs/kg feed)	Initial[b]	Breeding[b]	Whelping[b]	Three Weeks Post-Whelping[b]	Six Weeks Post-Whelping[b]	Necropsy[b]
Control	1205.8 ± 57.8	1207.1 ± 54.8	1225.9 ± 66.1	1035.4 ± 63.4	931.6 ± 52.8	894.6 ± 57.9
0.83	1247.1 ± 57.8	1237.4 ± 54.8	1326.9 ± 70.1	1113.3 ± 67.3	979.6 ± 56.0	952.7 ± 55.0
1.05	1250.3 ± 57.8	1249.4 ± 54.8	1303.3 ± 62.7	1156.4 ± 63.4	1041.6 ± 56.0	944.6 ± 61.5
1.69	1262.1 ± 57.8	1254.4 ± 54.8	1315.6 ± 66.1	1144.3 ± 63.4	987.3 ± 52.8	956.8 ± 52.4

[a] Data are presented as the mean ± standard error of the mean.

[b] The trial started on 2/11/02, breeding occurred 3/1/02 to 3/18/02, whelping occurred 4/26/02 to 5/7/02, and the adult female mink were necropsied during 6/17/02 to 7/8/02.

Table 7. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Body Weights (g) of Male Kits from Birth to 27 Weeks of Age[a]

Dietary Treatment (mg total PCBs/kg feed)	Birth	Three Weeks of Age	Six Weeks of Age	Necropsy at Six Weeks of Age	Necropsy at 27 Weeks of Age
Control	9.9 ± 0.3	105.7 ± 3.6	332.4 ± 12.5	428.2 ± 80.0	1713.7 ± 151.2
0.83	12.0 ± 0.4	115.5 ± 4.2	324.9 ± 13.6	368.0 ± 80.0	2049.5 ± 131.0
1.05	11.0 ± 0.4	123.5 ± 4.4	345.1 ± 14.2	354.9 ± 65.3	2188.0 ± 185.2
1.69	10.8 ± 0.4	111.1 ± 4.3	309.0 ± 13.9	357.1 ± 65.3	1815.3 ± 131.0

[a] Data are presented as the mean ± standard error of the mean. The end of the trial occurred on 11/5/02 resulting in approximately 27 weeks of exposure.

Table 8. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Body Weights (g) of Female Kits from Birth to 27 Weeks of Age[a]

Dietary Treatment (mg total PCBs/kg feed)	Birth	Three Weeks of Age	Six Weeks of Age	Necropsy at Six Weeks of Age	Necropsy at 27 Weeks of Age
Control	9.7 ± 0.4	94.3 ± 3.9	275.5 ± 12.2	295.1 ± 39.8	1180.3 ± 92.4
0.83	10.6 ± 0.4	105.1 ± 3.6	277.9 ± 10.8	295.0 ± 34.5	1385.5 ± 92.4
1.05	10.1 ± 0.3	112.6 ± 3.4	283.7 ± 10.3	296.4 ± 39.9	1228.0 ± 82.7
1.69	10.5 ± 0.3	101.3 ± 3.2	281.6 ± 9.6	276.5 ± 39.8	1219.3 ± 92.4

[a] Data are presented as the mean ± standard error of the mean. The end of the trial occurred on 11/5/02 resulting in approximately 27 weeks of exposure.

Table 9. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Absolute Organ Weights (g) of Adult Female Mink[a]

Dietary Treatment (mg total PCBs/kg feed)	Brain	Liver	Spleen	Kidneys	Heart	Adrenal Glands
Control	8.4 ± 0.23	40.4 ± 2.3	2.6 ± 0.7[a]	7.6 ± 0.4	5.6 ± 0.3	0.09 ± 0.01
0.83	7.9 ± 0.22	42.3 ± 2.2	5.2 ± 0.7[b]	7.6 ± 0.4	6.1 ± 0.3	0.08 ± 0.01
1.05	7.9 ± 0.23	44.3 ± 2.3	4.8 ± 0.7[ab]	7.4 ± 0.4	6.5 ± 0.3	0.07 ± 0.00
1.69	7.7 ± 0.22	41.9 ± 2.2	4.8 ± 0.7[ab]	7.7 ± 0.4	6.5 ± 0.3	0.07 ± 0.01

[a]Data are presented as the mean ± standard error of the mean. Means with different superscripts are significantly different from one another at $p<0.05$.

Table 10. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Relative Organ Weights (%) of Adult Female Mink[a]

Dietary Treatment (mg total PCBs/kg feed)	Brain	Liver	Spleen	Kidneys	Heart	Adrenal Glands
Control	0.95 (0.83 – 1.1)	4.6 (4.0- 5.2)	0.29[a] (0.20 – 0.41)	0.86 (0.75 – 0.97)	0.64 (0.56 – 0.73)	0.010 (0.008 – 0.013)
0.83	0.84 (0.73 – 0.96)	4.5 (4.0 – 5.1)	0.52[b] (0.40 – 0.66)	0.81 (0.71 – 0.91)	0.65 (0.57 – 0.73)	0.008 (0.006 – 0.010)
1.05	0.87 (0.75 – 1.0)	4.8 (4.2 – 5.4)	0.49[ab] (0.36 – 0.63)	0.81 (0.71 – 0.92)	0.70 (0.62 – 0.79)	0.008 (0.006 – 0.010)
1.69	0.82 (0.71 – 0.94)	4.4 (3.9 – 5.0)	0.49[ab] (0.37 – 0.62)	0.81 (0.72 – 0.91)	0.68 (0.61 – 0.77)	0.007 (0.006 – 0.010)

[a]Data are presented as the mean (95% confidence intervals). Relative organ weights are expressed as a percentage of body weight. Means with different superscripts are significantly different from one another at $p<0.05$.

Table 11. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Absolute Organ Weights of Kits Necropsied at Six Weeks of Age.[a]

Dietary Treatment (mg total PCBs/kg feed)	Brain	Liver	Spleen	Kidneys	Heart	Adrenal Glands	Thyroid Gland
Control	9.0 ± 0.5	17.7 ± 1.9	2.5 ± 0.3	3.3 ± 0.3	2.2 ± 0.2	0.04 ± 0.004	0.03 ± 0.006
0.83	9.0 ± 0.5	17.5 ± 2.0	1.7 ± 0.3	3.1 ± 0.4	2.0 ± 0.2	0.04 ± 0.004	0.03 ± 0.006
1.05	9.3 ± 0.5	19.6 ± 1.9	2.0 ± 0.3	3.0 ± 0.3	1.9 ± 0.2	0.03 ± 0.004	0.03 ± 0.006
1.69	9.4 ± 0.5	19.3 ± 1.9	2.2 ± 0.3	3.3 ± 0.3	2.0 ± 0.2	0.04 ± 0.004	0.03 ± 0.006

[a]Data are expressed as the mean ± standard error of the mean.

Table 12. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Relative Organ Weights (%) of Kits Necropsied at Six Weeks of Age.[a]

Dietary Treatment (mg total PCBs/kg feed)	Brain	Liver	Spleen	Kidneys	Heart	Male Adrenal Glands	Female Adrenal Glands	Thyroid Gland
Control	2.6 (2.2 – 3.1)	5.0 (4.4 – 5.6)	0.70 (0.58 – 0.83)	0.97 (0.89 – 1.05)	0.64 (0.58 – 0.70)	0.013 (0.010 – 0.016)	0.012 (0.009 – 0.014)	0.007 (0.004 – 0.011)
0.83	2.8 (2.4 – 3.3)	5.4 (4.9 – 6.0)	0.51 (0.42 – 0.62)	0.94 (0.86 – 1.02)	0.64 (0.58 – 0.70)	0.010 (0.008 – 0.013)	0.010 (0.008 – 0.013)	0.011 (0.007 – 0.015)
1.05	2.9 (2.5 – 3.3)	6.1 (5.5 – 6.7)	0.61 (0.51 – 0.72)	0.93 (0.86 – 1.00)	0.58 (0.53 – 0.63)	0.008 (0.006 – 0.010)	0.008 (0.006 – 0.010)	0.008 (0.005 – 0.011)
1.69	3.0 (2.6 – 3.4)	6.1 (5.5 – 6.7)	0.67 (0.57 – 0.79)	1.0 (0.96 – 1.1)	0.63 (0.58 – 0.69)	0.013 (0.010 – 0.015)	0.013 (0.010 – 0.015)	0.008 (0.005 – 0.011)

[a]Data are presented as mean (95% confidence interval). Relative organ weights are expressed as a percentage of body weight.

Table 13. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Absolute Organ Weights of Kits Necropsied at 27 Weeks of Age.[a]

Dietary Treatment (mg total PCBs/kg feed)	Brain	Liver	Spleen	Kidneys	Heart	Adrenal Glands	Thyroid Gland
Control	9.3 ± 0.4	61.0 ± 3.9	3.0 ± 0.5[a]	8.8 ± 0.6	7.5 ± 0.5	0.10 ± 0.01	0.06 ± 0.01
0.83	9.6 ± 0.3	67.1 ± 3.6	4.3 ± 0.5[ab]	9.5 ± 0.6	8.4 ± 0.4	0.11 ± 0.01	0.07 ± 0.01
1.05	9.3 ± 0.4	63.5 ± 4.3	3.4 ± 0.5[ab]	8.0 ± 0.7	8.0 ± 0.5	0.09 ± 0.01	0.06 ± 0.01
1.69	10.0 ± 0.3	62.8 ± 3.6	5.1 ± 0.5[b]	8.2 ± 0.6	8.5 ± 0.4	0.10 ± 0.01	0.06 ± 0.01

[a]Data are expressed as mean ± standard error of the mean. Means with different superscripts are significantly different from one another at $p<0.05$.

Table 14. The Effect of PCBs/PCDDs/PCDFs Derived From Saginaw River Fish on Relative Organ Weights (%) of Kits Necropsied at 27 Weeks of Age.[a]

Dietary Treatment (mg total PCBs/kg feed)	Brain	Liver	Spleen	Kidneys	Heart	Adrenal Glands	Thyroid Gland
Control	0.65 (0.56 – 0.75)	4.2 (4.0 – 4.5)	0.21[a] (0.16 – 0.27)	0.61 (0.54 – 0.68)	0.51 (0.46 – 0.56)	0.007 (0.006 – 0.008)	0.004 (0.003 – 0.005)
0.83	0.58 (0.50 – 0.67)	3.9 (3.7 – 4.2)	0.25[ab] (0.20 – 0.31)	0.55 (0.54 – 0.61)	0.49 (0.44 – 0.53)	0.006 (0.006 – 0.007)	0.004 (0.003 – 0.005)
1.05	0.59 (0.49 – 0.69)	3.8 (3.6 – 4.2)	0.21[ab] (0.15 – 0.28)	0.54 (0.46 – 0.60)	0.48 (0.42 – 0.53)	0.006 (0.005 – 0.007)	0.004 (0.003 – 0.005)
1.69	0.67 (0.59 – 0.77)	4.2 (3.9 – 4.4)	0.34[b] (0.28 – 0.41)	0.52 (0.47 – 0.58)	0.56 (0.51 – 0.61)	0.007 (0.006 – 0.007)	0.004 (0.003 – 0.005)

[a]Data are presented as the mean (95% confidence interval). Relative organ weights are expressed as a percentage of body weight. Means with different superscripts are significantly different from one another at $p<0.05$.

Table 15. The Histopathological Assessment of Squamous Cell Proliferation in the Maxillae and Mandibles from Mink Kits Exposed to PCBs/PCDDs/PCDFs Derived from Saginaw River Fish Through 27 Weeks of Age.

Animal ID	Dietary Treatment (mg total PCBs/kg feed)	Observation on Occurrence and Numbers of Foci of Squamous Cell Proliferation
1390-M1-30	Control	No lesions
600-M1-30	Control	No lesions
842-F1-30	Control	No lesions
872-F1-30	Control	No lesions
964-F1-30	Control	No lesions
990-F1-28	Control	No lesions
990-M1-30	Control	No lesions
754-F1-30	0.83	No lesions
754-M1-30	0.83	No lesions
772-F1-30	0.83	No lesions
772-M1-30	0.83	No lesions
830-F1-28	0.83	No lesions
830-M1-28	0.83	No lesions
870-F1-30	0.83	No lesions
916-M1-30	0.83	No lesions
1294-M1-30	1.05	No lesions
1392-M1-30	1.05	No lesions
1400-F1-28	1.05	Mandible, 1 focus, mild
1400-F2-28	1.05	Maxilla, 2 foci, mild
614-F1-30	1.05	Mandible, 1 focus, mild
974-F1-30	1.05	No lesions
974-F2-30	1.05	Mandible, 2 foci, mild
1010-F1-30	1.69	Mandible, 1 focus, mild
1410-F1-30	1.69	Mandible, 2 foci, mild to moderate Maxilla, 2 foci, mild to moderate
1410-M1-30	1.69	Mandible, 5 foci, moderate Maxilla, 2 foci, moderate
606-M1-28	1.69	Mandible, 2 foci, moderate Maxilla, 8 foci, moderate
700-F1-30	1.69	No lesions
700-M1-30	1.69	Mandible, 1 focus, mild Maxilla, 1 focus, mild
714-F1-28	1.69	No lesions
930-M1-30	1.69	Mandible, 1 focus, mild to moderate Maxilla, 2 foci, mild to moderate

Table 16. The Effect of PCB 126 and TCDD on the Number of Female Mice with Litters, Litter Size and Pup Survivability Through 21 Days of Age.

Dietary Treatment	Number of Females Delivering/ Total Number of Females	Average Litter Size[a]	% Survivability[b]
Control	8/8	12.1 ± 1.0	93.5 (67.6 – 99.2)
5.0 µg PCB 126/kg feed	4/4	14.8 ± 1.5	89.3 (46.0 – 97.5)
10 µg PCB 126/kg feed	4/4	12.8 ± 1.5	59.4 (14.0 – 96.3)
20 µg PCB 126/kg feed	3/4	12.7 ± 1.7	81.6 (27.6 – 98.3)
0.5 µg TCDD/kg feed	4/4	14.8 ± 1.5	75.3 (28.0 – 100.0)
1.0 µg TCDD/kg feed	3/4	11.3 ± 1.7	94.1 (47.1 – 89.9)
2.0 µg TCDD/kg feed	4/4	11.5 ± 1.5	41.6 (4.2 – 86.4)

[a]Data are expressed as mean ± standard error of the mean. [b]Data are expressed as mean (95% confidence interval).

Table 17. The Histopathological Assessment of Maxillae and Mandibles from Mice Exposed to PCB 126 or TCDD From Conception Through Time of Necropsy

Animal ID	Age (Days)	Dietary Treatment	Observation on Occurrence and Numbers of Foci of Epithelial Cell Aggregates
C2-04-D21	21	Control	No histological alterations
C3-02-D21	21	Control	No histological alterations
C4-01-D21	21	Control	No histological alterations
C3-01-D42	42	Control	No histological alterations
C4-02-D70	70	Control	No histological alterations
C2-01-D98	98	Control	No histological alterations
C4-02-D98	98	Control	No histological alterations
C9-01-D21	21	5.0 µg PCB126/kg feed	No histological alterations
C10-02-D21	21	5.0 µg PCB126/kg feed	No histological alterations
C10-03-D21	21	5.0 µg PCB126/kg feed	No histological alterations
C12-02-D28	28	5.0 µg PCB126/kg feed	No histological alterations
C9-01-D84	84	5.0 µg PCB126/kg feed	No histological alterations
C10-02-D98	98	5.0 µg PCB126/kg feed	No histological alterations
C13-04-D21	21	10 µg PCB126/kg feed	No histological alterations
C14-02-D21	21	10 µg PCB126/kg feed	No histological alterations
C13-02-D42	42	10 µg PCB126/kg feed	No histological alterations
C20-04-D21	21	20 µg PCB126/kg feed	2 minimal foci of epithelial cell aggregates in periodontal ligament of 2 molar teeth
C18-02-D42	42	20 µg PCB126/kg feed	No histological alterations
C20-01-D56	56	20 µg PCB126/kg feed	4 minimal foci of epithelial cell aggregates in periodontal ligament of 2 molar teeth
C17-01-D84	84	20 µg PCB126/kg feed	3 minimal foci of epithelial cell aggregates in periodontal ligament of 2 molar teeth
C20-02-D84	84	20 µg PCB126/kg feed	No histological alterations
C21-01-D21	21	0.5 µg TCDD/kg feed	No histological alterations
C21-04-D21	21	0.5 µg TCDD/kg feed	No histological alterations
C22-01-D84	84	0.5 µg TCDD/kg feed	No histological alterations
C24-01-D84	84	0.5 µg TCDD/kg feed	2 minimal foci of epithelial cell aggregates in periodontal ligament of 1 molar tooth
C25-04-D21	21	1.0 µg TCDD/kg feed	No histological alterations
C25-05-D21	21	1.0 µg TCDD/kg feed	No histological alterations
C26-02-D28	28	1.0 µg TCDD/kg feed	1 minimal foci of epithelial cell aggregate in periodontal ligament of 1 molar tooth
C28-01-D42	42	1.0 µg TCDD/kg feed	No histological alterations
C26-02-D42	42	1.0 µg TCDD/kg feed	2 minimal foci of epithelial cell aggregates in periodontal ligament of 1 molar tooth
C26-02-D84	84	1.0 µg TCDD/kg feed	No histological alterations

Table 17 continued. The Histopathological Assessment of Maxillae and Mandibles from Exposed to PCB 126 or TCDD From Conception Through Time of Necropsy			
Animal ID	Age (Days)	Dietary Treatment	Observation on Occurrence and Numbers of Foci of Epithelial Cell Aggregates
C30-02-D21	21	2.0 µg TCDD/kg feed	No histological alterations
C32-02-D21	21	2.0 µg TCDD/kg feed	No histological alterations
C32-03-D21	21	2.0 µg TCDD/kg feed	No histological alterations
C29-01-D28	28	2.0 µg TCDD/kg feed	4 minimal foci of epithelial cell aggregates in periodontal ligament of 1 molar tooth
C30-02-D42	42	2.0 µg TCDD/kg feed	8 minimal foci of epithelial cell aggregates in periodontal ligament of 3 molar teeth
C29-02-D56	56	2.0 µg TCDD/kg feed	3 minimal foci of epithelial cell aggregates in periodontal ligament of 2 molar teeth
C29-01-D84	84	2.0 µg TCDD/kg feed	No histological alterations